HIV-related neutropenia and endogenous G-CSF production: Do severely depressed serum G-CSF levels correlate with a favorable response to recombinant G-CSF administration?

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A. Study Purpose and Rationale

Neutropenia (defined as an absolute neutrophil count, ANC < 1000/□l) occurs frequently in the setting of HIV infection, with prevalence estimates ranging from 10-40% [1, 2], and is multifactorial in etiology. The principal precipitants of neutropenia in this setting are thought to be: 1) HIV infection of hematopoeitic progenitor cells, 2) myelosuppressive drugs such as zidovudine, sulfamethoxazole and ganciclovir, 3) intercurrent myeloinfiltrative processes such as HIV-related lymphoma and infection with cytomegalovirus or *Mycobacterium avium* complex, 4) nutritional deficiency (vitamin B12) and 5) antibodies to gp120, which suppress bone marrow progenitors [3].

In addition, neutrophil function is impaired in advanced HIV infection; this is characterized by accelerated apoptosis [4], diminished fungicidal and bactericidal activity [5], and impaired secretion of the cytokine, granulocyte-colony stimulating factor (G-CSF). HIV-related neutropenia is clinically significant and has been shown to increase the risk for secondary infectious complications, especially when associated with a low CD4 count [6, 7].

Several randomized clinical trials have demonstrated the therapeutic benefit of recombinant G-CSF in HIV patients, in order to prevent severe neutropenia [8], and especially in conjunction with ganciclovir for CMV infection [9]. One study showed that G-CSF improved oxidative capacity and bactericidal activity, while decreasing the frequency of apoptosis in neutrophils [10]. For this reason, many clinicians have opted to treat HIV patients who develop febrile neutropenia with a course of recombinant G-CSF, titrated to the ANC response.

However, recombinant G-CSF is expensive and severe adverse reactions such as disseminated intravascular coagulation and pancreatitis have been reported [11, 12]. In addition, controversy exists as to the clinical context where G-CSF therapy is indicated. Unless they are exposed to ganciclovir or chemotherapy for lymphoma, HIV patients tend to maintain ANCs above 500/□l, and are thus at lower risk for life-threatening bacterial or fungal sepsis.

A striking variability in the response to G-CSF therapy, as measured by fold-change in ANC, has been reported by Nielsen and colleagues [13]. In this study, the median response to G-CSF treatment resulted in a 4-fold increase in the ANC. However, the standard deviation from the mean was of equivalent magnitude. This observation suggests that some patients are not responding at all, while others suffer from a relative deficiency of this cytokine, and therefore efficiently generate neutrophils when treated. This variability in the response to exogenous G-CSF may be attributed to the multifactorial nature of neutropenia in HIV, which in turn may be related to endogenous G-CSF production.

Baseline levels of endogenous G-CSF have been measured in HIV patients with afebrile neutropenia and compared with HIV patients with pneumonia. Interestingly, afebrile neutropenic HIV patients generate very low serum levels of G-CSF (< 100 pg/ml, median < 10 pg/ml), similar to healthy control subjects, whereas HIV patients challenged with an acute lower respiratory tract infection had G-CSF levels ranging up to 2000 pg/ml (median 152 pg/ml) [1].

Hypothesis: A subset of neutropenic HIV patients is severely deficient in endogenous G-CSF levels, and is therefore more likely to respond favorably to exogenous administration of recombinant G-

CSF. We predict that the magnitude of change in absolute neutrophil count after therapy will be greatest in HIV patients with the lowest initial endogenous G-CSF level.

B. Study Design and Statistical Analysis

The study design is an observational, prospective cohort study. Patients will be divided into 2 groups, based on their baseline endogenous G-CSF level (< 200 pg/ml and > 200 pg/ml), and will be followed prospectively during and after G-CSF therapy. ANC determinations at weekly intervals will be plotted, and the fold-change in ANC before and at steady state after therapy will be calculated for each patient. We expect a 4-fold change in ANC overall, based on prior studies of G-CSF response [13], with high-responders generating a median 5-fold increase, and low-responders a 3-fold increase in ANC. Statistical analysis will be performed with the unpaired t-test, powered to detect a 2-fold difference between the two patient groups. We therefore would need to recruit 130 patients, 65 patients in each group. Sample size calculation: $n = 1 + 16 (\text{std-dev/effect})^2$.

In order to assess other factors involved in neutropenia, multiple regression analysis of fold-change in ANC will be performed on: baseline ANC, CD4 count, HIV viral load, myelosuppressive medications (zidovudine, sulfamethoxazole, ganciclovir), and concomitant myeloinfiltrative processes (CMV, MAC, lymphoma).

C. Study Procedure

Serum levels of endogenous G-CSF will be measured by ELISA in the ICCR Core Laboratory, the lower limit of detection in published reports is 10 pg/ml [13]. Serum samples will be drawn at the time of enrollment and prior to the initiation of recombinant G-CSF therapy. Baseline complete blood counts with ANC determinations will be recorded as well. The decision to treat with G-CSF, and duration of treatment (typically 7-10 days) will be at the discretion of the primary physician. The primary outcome variable, absolute neutrophil count, will be assayed once weekly for 4 weeks in each patient, in accordance with standard clinical care. Upon discharge, patients who have not completed 4 weeks of monitoring will be given a laboratory test slip for an outpatient complete blood count. Patients are therefore unlikely to experience any additional pain, discomfort or inconvenience as a result of this study. The expected duration of the study is 12 months, with each subject participating for 4 weeks.

D. Study Drugs

Recombinant human G-CSF (filgastrim) is approved as a safe and efficacious treatment for AIDS neutropenia and is available on the hospital formulary. It is administered once daily as a subcutaneous injection, 1-10 mcg/kg/day; approximate cost is $175.00 per 300 mcg dose, $300.00 per 480 mcg dose. Anaphylaxis and thrombocytopenia are known serious adverse reactions. In addition, common side-effects include bone pain, nausea, vomiting, musculoskeletal pain, rash, local skin reactions at the injection site, and hypotension.

E. Medical Devices

None.

F. Study Questionnaires

None.

G. Study Subjects

a. Inclusion criteria

patients with documented HIV infection, followed in the Harkness-6 Clinic or admitted to the AIDS/TB service, who develop febrile neutropenia and whose primary physicians have decided to begin G-CSF therapy. For each patient, most recent CD4 count and viral load, as well as concurrent medications such as zidovudine, ganciclovir, trimethoprim/sulfamethoxazole will be scored. If documented by bone marrow biopsy, presence of intercurrent bone marrow infiltrative processes such as lymphoma, CMV or MAC will also be recorded.

b. Exclusion criteria

age < 18 years, concurrent chemotherapy treatment for a malignancy, and renal failure.

H. Recruitment of Subjects

Patients followed in the HP-6 Clinic who develop neutropenia will be identified by their clinic physicians, patients admitted to the AIDS/TB service will be identified by the house staff caring for them. If the primary physician agrees that the patient is suitable for the study, then the study investigator will discuss its purpose, risks and benefits, and obtain written informed consent from those patients willing to participate.

I. Confidentiality of Study Data

Subjects will be assigned a code number and all data will be kept in a locked, secure location, accessible only to the study investigators.

J. Potential Conflict of Interest

None of the investigators have a financial interest in Amgen, which manufactures filgastrim, nor do they serve on any advisory boards affiliated with this pharmaceutical company.

K. Location of the Study

Harkness-6 Clinic and AIDS/TB inpatient service at New York-Presbyterian Hospital, Columbia Campus.

L. Potential Risks

None specific to this observational study.

M. Potential Benefits

Patients participating in this study may or may not benefit, as they will be treated according to the standard of clinical care. Ultimately they may benefit as a group, since the study addresses the question of clinical criteria for G-CSF therapy.

N. Alternative Therapies

Not applicable.

O. Compensation to Subjects

None.

P. Costs to Subjects

None, as cost of standard clinical therapy, including filgastrim is covered by Medicaid.

Q. Minors as Research Subjects

Not applicable.

R. Radiation or Radioactive Substances

Not applicable.

S. Literature References

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